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# Supercritical Fluid Extraction of Fat from Ground Beef: Effects of Water on Gravimetric and GC-FAME Fat Determinations

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This study investigated the supercritical carbon dioxide (SC-CO<sub>2</sub>) extraction of fat from ground beef and the effects of several factors on the gravimetric determination of fat. The use of ethanol modifier with the SC-CO<sub>2</sub> was not necessary for efficient fat extraction; however, the ethanol did increase the coextraction of water. This coextraction of water caused a significant overestimation of gravimetric fat. Oven-drying ground beef samples prior to extraction inhibited the subsequent extraction of fat, whereas oven-drying the extract after collection decreased the subsequent gas chromatographic fatty acid methyl ester (GC-FAME) fat determination. None of the drying agents tested were able to completely prevent the coextraction of water, and silica gel and molecular sieves inhibited the complete extraction of fat. Measurements of collection vial mass indicated that CO<sub>2</sub> extraction/collection causes an initial increase in mass due to the density of CO<sub>2</sub> (relative to displaced air) followed by a decrease in vial mass due to the removal of adsorbed water from the collection vial. Microwave-drying of the empty collection vials removes ~3 mg of adsorbed water, ~15–20 min is required for readsorption of the displaced water. For collection vials containing collected fat, microwave-drying effectively removed coextracted water, and the vials reached equilibration after ~10–15 min. Silanizing collection vials did not significantly affect weight loss during microwave-drying. SC-CO<sub>2</sub> can be used to accurately determine fat gravimetrically for ground beef, and the presented method can also be followed by GC-FAME analysis to provide specific fatty acid information as well.

**Keywords:** *Supercritical fluid extraction; fat; ground beef; water content; fatty acid methyl ester; gravimetric; carbon dioxide*

## INTRODUCTION

Although the determination of fat content is one of the most common analyses performed in a food laboratory, the quantitative extraction and analysis of fat is far from straightforward (1). Historically, many methods determine fat content by gravimetric measurements. Although these methods have been in use for a long time, their accuracy is questionable because they are not always specific for fat. In addition, they do not provide any information on the types of fats present (e.g., saturated or unsaturated fat). These shortcomings were addressed by the Nutritional Labeling and Education Act (NLEA) of 1990 (2), and total fat is currently defined as the sum of all fatty acids obtained from total lipid extract expressed as triglycerides. The NLEA protocol consists of the following steps: (1) a hydrolytic treatment of the sample, (2) solvent extraction of lipids, and (3) preparation of fatty acid methyl esters (FAMES) for gas chromatographic (GC) analysis and quantitation of saturated and unsaturated fat after stoichiometric conversion of FAMES to triglycerides. Although such GC-FAME methods are very accurate, they are somewhat tedious and time-consuming to perform. This type of analysis must be performed to satisfy NLEA requirements for food labeling; however, there are instances when gravimetric fat determinations are sufficient. For

example, in-house quality control and assurance programs may not need GC-FAME analyses to know that the product meets given specifications. For example, fresh meat products such as ground beef do not require that the fat content be separated into saturated and unsaturated fats as do other food products. The Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA) currently requires only total fat be given on products such as ground beef, in which a gravimetric determination will suffice to determine fat content.

Supercritical fluid extraction (SFE) is a practical means to extract and subsequently determine the fat content of foods. Fat recoveries for SFE and solvent-based extraction methods are generally in good agreement (3), and the precision of analytical SFE is comparable to that of traditional organic solvent-based methods, and in some cases better (4). The use of supercritical CO<sub>2</sub> (SC-CO<sub>2</sub>) as an extraction medium has many advantages. Carbon dioxide is nontoxic, noncombustible, inexpensive (5), and easily removed from the extract, and there are no costs associated with solvent waste disposal (6). SC-CO<sub>2</sub> methods can also result in reduced extraction times, and they can be automated (7). Previously, our laboratory used SFE to extract fat and compare gravimetric and GC-FAME fat determinations for a variety of foods, including oilseeds, meat products, bakery products, and a NIST standard reference material (8, 9). During analyses of ground beef samples, it was found that the disparity between the gravimetric

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(13.3%) and subsequent GC-FAME determination (12.0%) was highest for the sample with the lowest fat content (9). We hypothesized that this disparity of percent fat content was due to the coextraction of water with the fat by the SC-CO<sub>2</sub>. As the fat content of ground beef decreases, the water content increases more quickly than does the protein content (10, 11). For regular ground beef, the proximate values of fat, water, and protein are about 26, 55, and 17%, respectively, whereas for extra-lean ground beef, they are about 16, 64, and 19%, respectively (12, 13).

The selective extraction of only the analyte of interest is relatively rare and, in general, the analyte of interest is coextracted with interfering compounds (14). Water is soluble in SC-CO<sub>2</sub>, and at 100 °C and 9000 psi it is ~3 mol % (15). At this solubility level, a 30 min extraction and a flow rate of 2 L/min expanded CO<sub>2</sub>, the SC-CO<sub>2</sub> could solubilize ~1.4 g of water. Previously, the extraction of high amounts of water as well as triglycerides from ground beef has been reported (16). The contribution of nonfat materials can become a serious issue in low-fat products when using a gravimetric fat method (17). For example, 10 mg of coextracted water counted as fat would cause a 20% overestimation of the actual fat content for a 1 g sample containing 5% fat (i.e., 50 mg of fat).

The purpose of this research was to study the SC-CO<sub>2</sub> extraction of fat from ground beef in an attempt to develop a more accurate SC-CO<sub>2</sub> gravimetric extraction method for determining fat levels for ground beef. We examined the effects of ethanol modifier and sample drying, the effect of drying agents inside the extraction cell, the effect of postextraction equilibration time, the effect of microwave-drying of collection vials, and the effect of silanizing collection vials on microwave-drying of collection vials.

## MATERIALS AND METHODS

**Ground Beef Samples.** The ground beef samples (nominally 10% fat) were prepared by the Department of Animal Science at the University of Illinois, Urbana, IL (4). The samples were vacuum-sealed, frozen, and held at -20 °C until used for evaluation.

**Supercritical Fluid Extraction Procedure.** Supercritical fluid extractions were conducted with a Leco Corp. model FA-100 SFE (Leco Corp. St. Joseph, MI). Approximately 1 g of sample was weighed to the nearest 0.0001 g in a 50-mL beaker and mixed with ~1.5 g of Leco-Dry (Leco Corp.) (three scoops using Leco glass scoop, part 776-978). Leco Dry is a type of diatomaceous earth used to absorb water, disperse the sample, make a free-flowing mixture, and reduce solvent channeling through the extraction cell (18, 19). This mixture was then added to the extraction thimble containing a glass fiber filter disk (8 mm diameter, Leco Corp.) and ~0.5 g of Leco-Dry (one scoop) on the bottom. Sufficient Leco-Dry was added to nearly fill the thimble, and a second glass fiber filter was placed on top. SFE was performed at 9000 psi and 100 °C at a flow rate of 2 L/min for 25 min after an initial 5 min static hold. The variable restrictor was heated to 100 °C. Although Leco suggests that collection vials be packed with glass wool, because of the difficulties in removing the collected fat from the glass wool and to facilitate the subsequent transesterification and GC analysis, some collections were performed in vials containing ~5 g of glass helices (0.5 mm gauge, 4.2 mm diameter helix) (9). SFE/SFC grade CO<sub>2</sub> (Air Products and Chemicals, Inc., Allentown, PA) was used for all SFE experiments.

**Gravimetric Fat Determination and Transesterification.** The collected fat was weighed, and the gravimetric percentage fat was determined on the basis of the weight of

original sample. GC-FAME fat determinations were performed according to the general procedure described by House et al. (20). One milliliter of a 10.00 mg/mL solution of triundecanoin in toluene was added to the collected fat residue on the glass helices along with 2 mL of 7% BF<sub>3</sub> in methanol. The vial was sealed with a Teflon-lined screwcap and heated to 100 °C for 45 min with gentle mixing every 10 min. The vial was then allowed to cool to room temperature, and 5 mL of deionized water, 1 mL of hexane, and ~1 g of Na<sub>2</sub>SO<sub>4</sub> were added and mixed vigorously. The vial was centrifuged to separate the layers, and the top layer was removed for subsequent GC-FAME analysis.

**Acid Hydrolysis (AH) and Solvent Extraction.** The fat level of the ground beef was established using AOAC Method Am 3-96 (21). One milliliter of a 10.00 mg/mL solution of triundecanoin in chloroform was added to a 50 mL glass-stoppered Erlenmeyer flask, and the solvent was evaporated under a gentle stream of nitrogen. Approximately 1 g of sample was weighed to the nearest 0.0001 g into the flask, and ~100 mg of pyrogallol, 1 mL of EtOH, and 5 mL of 8.3 N HCl were added to the flask. The flask was stoppered and placed in a shaker bath set at 80 °C and 150 rpm. After 40 min, the flask was removed, allowed to cool to room temperature, and extracted with 25 mL of diethyl ether and 25 mL of hexane. The combined ether/hexane extracts were evaporated under nitrogen, and the residue was extracted with 5 mL of chloroform and transferred to a 12 mL screwcap vial. The chloroform extract was evaporated under nitrogen, and the residue was transesterified (using toluene without triundecanoin) and analyzed by GC as described above.

**GC Analysis and Quantification of Fat.** Fat determination by GC-FAME analysis was performed according to the method of King et al. (4). FAMES were analyzed by split injection (200:1 split ratio) onto a Hewlett-Packard series II GC equipped with a flame ionization detector. The column used was an SP-2340 (60 m, 0.25 mm diameter, 0.20 µm film thickness) (Supelco, Bellefonte, PA) with He as the carrier gas at a linear flow velocity of 18 cm/s. The temperature program was 100 °C for 5 min, 3 °C/min to 190 °C, 1 °C/min to 200 °C, held for 15 min, then 50 °C/min to 250 °C, and held for 1 min. The injector and detector temperatures were 235 and 250 °C, respectively. Injections were made using a Hewlett-Packard 7683 autoinjector, and the sample volume was 1 µL. Chromatographic data were acquired using a Hewlett-Packard Vectra VL2 computer and ChemStation software. The weights of the individual FAMES were calculated on the basis of their integrations relative to the triundecanoin internal standard and were corrected using corresponding GC response factors for each fatty acid (20). The weights of the individual FAMES were converted to equivalent weights of triglycerides using appropriate conversion factors (17). Total fat was calculated as the sum of all fatty acids expressed as triglycerides.

**Effect of Ethanol Modifier and Sample Drying.** In a previous study in which ethanol was used as a modifier, there was a significant difference between the gravimetric and the subsequent GC-FAME fat determinations (13.3 and 12.0% fat, respectively) for the ground beef sample with the lowest fat level (i.e., nominally 10%), whereas the gravimetric and GC-FAME determinations for the nominally 20 and 30% fat ground beef samples were statistically equivalent (9). The purpose of this experiment was to determine if the ethanol added as a modifier increased the extraction of nonfat material such as water and if ethanol was necessary for complete extraction of fat from ground beef. In addition, the effects of drying the sample before extraction and drying the extract after collection were studied. Although it is possible to remove moisture from samples by freeze-drying, this method is time-consuming (18) and we chose to use a vacuum oven instead. All possible combinations with and without modifier and drying (i.e., no drying, drying before extraction, and drying after collection) were tested. In addition, both gravimetric and GC-FAME determinations were made for all modifier/drying combinations for a total of 12 treatments (Table 1). The general SFE procedure outlined above was used, except for the ethanol modifier treatments (i.e., CO<sub>2</sub> with EtOH), which consisted of

**Table 1. Effect of Sample Drying (Vacuum Oven) and Ethanol Modifier on Mean<sup>a</sup> Percentage Fat**

drying method	CO <sub>2</sub> only		CO <sub>2</sub> /EtOH	
	gravimetric	GC-FAME	gravimetric	GC-FAME
no drying	11.8 cde	11.7 de	13.5 a	12.0 cd
dried before extraction	11.2 fg	11.1 g	12.2 c	11.5 ef
dried after collection	11.8 cde	11.5 efg	13.0 b	11.7 de

<sup>a</sup> ( $n = 6$ ), means without letters in common differ significantly (linear contrast  $t$  test).

adding 1 mL of absolute ethanol on top of the filled extraction cell. Ground beef samples were dried before extraction by mixing with Leco-Dry and then drying for 30 min in a vacuum oven (~74 cm mercury vacuum) set at 100 °C and then placed inside the extraction thimbles. The extracted/collected material in the collection vials was dried in a similar manner.

**Effect of Drying Agents in the Extraction Cell.** In an effort to retain water inside the extraction cell that would otherwise be coextracted with the fat, several drying agents were added to the bottom of the extraction cell (flow through the cell is top to bottom) in place of the 1.5 g of Leco-Dry generally used on the bottom of the extraction thimble. The three drying agents used were anhydrous Na<sub>2</sub>SO<sub>4</sub>, silica gel, and 3 Å molecular sieves. Approximately 2.5 mL of each drying agent was used, and the approximate masses were 4.8, 1.5, and 2.3 g, respectively. Supercritical fluid extractions of 10% ground beef were done using neat CO<sub>2</sub>, glass helices were used in the collection vials, and both gravimetric and GC-FAME fat determinations were made.

**Effect of Postextraction Equilibration Time.** The time required for the mass of the collection vials to re-equilibrate after an extraction/collection was studied for extraction thimbles of cells filled with Leco-Dry only (i.e., "sham" extraction) using glass wool or glass helices in the collection vials. The time required for equilibration of collection vials containing glass helices after SC-CO<sub>2</sub> extraction of 10% ground beef was also examined. Extraction conditions were as described above, and the collection vials were weighed immediately after extraction and every 5 min up to 35 min postextraction.

**Effect of Microwave-Drying of Collection Vials.** The effect of microwave-drying of collection vials containing only glass wool or glass helices without collected fat was examined as well as vials containing glass helices with collected fat from the SFE of 10% fat ground beef. Collection vials were microwaved on "high" in a 700 W microwave (model MW8590T, Samsung Electronics, Ridgefield Park, NJ) for 3 min. The vials without collected fat were weighed prior to microwaving, microwaved, and weighed every 5 min up to 35 min postmicrowaving. The vials with the extracted/collected fat were weighed prior to the extraction/collection, immediately after collection, and 5 min postcollection; the vials were then microwaved and weighed every 5 min up to 35 min postmicrowaving. GC-FAME fat determinations were also made for collection vials that had been dried by microwaving as well as those without any drying to determine if microwave drying had any effect on the subsequent GC-FAME fat determination.

**Effect of Silanizing Collection Vials on Equilibration Time after Microwave-Drying.** In an effort to decrease the effect of microwave-drying on the loss of water from the collection vials, the collection vials were silanized on both the inside and outside using dichlorodimethylsilane (Aldrich, Milwaukee, WI). In addition, the glass helices used to fill the collection vials were silanized as well. Collection vials without collected fat were weighed prior to microwaving, microwaved, and weighed every 5 min up to 35 min postmicrowaving.

**Statistical Analyses.** Analyses of variance (ANOVA) were performed on percentage fat, after arcsin transformation (to stabilize variance) (22), using Statistix 4.1 software (Analytical Software, Tallahassee, FL). Means were compared using linear contrast  $t$  tests at the  $P = 0.05$  level.

**Table 2. Effect of Drying Agents in the Extraction Cell on Mean<sup>a</sup> Percentage Fat**

drying agent	fat determination method	
	gravimetric	GC-FAME
none	11.9 a	11.6 ab
Na <sub>2</sub> SO <sub>4</sub>	12.0 a	11.7 ab
silica gel	11.5 ab	11.2 bc
molecular sieve	10.5 c	9.8 d

<sup>a</sup> ( $n = 6$ ), means without letters in common differ significantly (linear contrast  $t$  test).

## RESULTS AND DISCUSSION

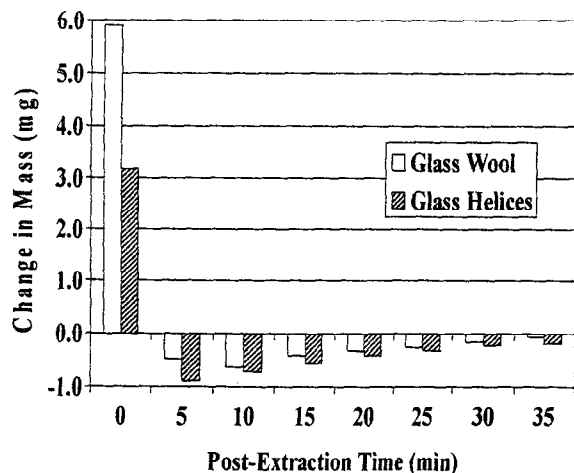
### Effect of Ethanol Modifier and Sample Drying.

The effects of ethanol modifier and sample drying are shown in Table 1. For all three drying treatments (i.e., no drying, drying before extraction, and drying after collection), the addition of ethanol modifier gave the highest gravimetric fat determinations. Moreover, when ethanol modifier was used, the differences between these gravimetric determinations and the subsequent GC-FAME determinations were highly significant, indicating that the ethanol modifier was extracting materials other than just fat. The difference between the gravimetric and GC-FAME determinations for the sample dried before extraction was less than the difference between the gravimetric and GC-FAME determinations for the extract dried after collection treatment. This suggests that SC-CO<sub>2</sub> modified with ethanol extracts more water than SC-CO<sub>2</sub> alone. For the sample without any drying, there was no significant difference between the GC-FAME determinations for the unmodified SC-CO<sub>2</sub> and the ethanol-modified SC-CO<sub>2</sub>, indicating that the ethanol does not improve extraction of fat from ground beef.

When the ground beef sample was dried before extraction and extracted with neat CO<sub>2</sub>, the gravimetric and subsequent GC-FAME determinations were essentially equal. However, this GC-FAME determination was significantly less than the GC-FAME determination of the treatment without any drying. Although King (14) reported that fats were more effectively extracted from meat samples air-dried at room temperature than wet samples because hydrophilic matrices inhibit contact between the supercritical fluid and the fat, vacuum oven-drying prior to extraction seems to somehow inhibit the subsequent extraction of fat by SC-CO<sub>2</sub>. Hagan et al. (23) also reported that the amount of lipids extracted is dependent on the drying method. This may be due to oxidation of unsaturated components (17) or possibly a result of polymerization of triglycerides (24). When the collected fat was dried after extraction, only when ethanol modifier had been used was there a significant decrease between the original gravimetric determination and the subsequent GC-FAME determination. This suggests that the ethanol modifier removes extraneous water during the extraction of fat from the ground beef.

### Effect of Drying Agents in the Extraction Cell.

The results of the drying agents on fat determination are shown in Table 2. The difference between the gravimetric and subsequent GC-FAME determinations when molecular sieves were used inside the extraction cell was the highest of any of the drying treatments tested and statistically significant. In addition, the GC-FAME determination for this treatment was significantly less than the GC-FAME determination for the treatment without drying agent. This suggests that

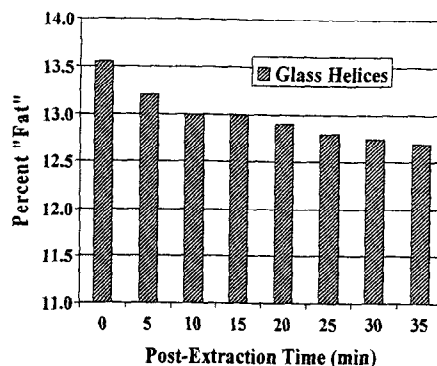


**Figure 1.** Effect of vial packing material and postextraction equilibration time on collection vial weight: sham extraction.

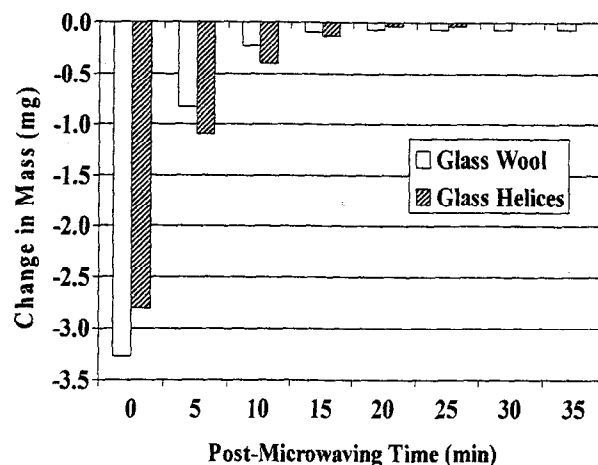
molecular sieves may have retained some fat in the extraction cell, resulting in an underestimation of the fat content. There were no statistical differences between the gravimetric and subsequent GC-FAME determinations for any of the other treatments, including the treatment without any drying agent added. Therefore, although neither silica gel nor  $\text{Na}_2\text{SO}_4$  had an adverse effect of fat determination, these results indicate that drying agents may be unnecessary for retaining water during the extraction of fat from ground beef in the absence of ethanol modifier.

**Effect of Postextraction Equilibration Time.** The change in mass of collection vials with postextraction time for the sham extraction (i.e., no ground beef in the extraction cell) is shown in Figure 1. The collection vials filled with glass wool had gained an average of just under 6 mg when weighed immediately after the extraction, whereas the vials filled with glass helices had gained an average of just over 3 mg. This increase in mass observed immediately after the extraction is undoubtedly a result of the collection vials being filled with expanded  $\text{CO}_2$  decompressed after the extraction. The density of  $\text{CO}_2$  is  $\sim 1.84$  mg/mL, and it is  $\sim 1.53$  times as dense as air (*Merck Index*). The volume of an empty collection vial is  $\sim 23$  mL; therefore, one would expect a vial filled with  $\text{CO}_2$  to weigh  $\sim 14.6$  mg more than a vial filled with air. Considering the fact that the vials contained glass wool or helices, decreasing their internal volumes and the potential diffusion of  $\text{CO}_2$  from the vials as they were transferred from the SFE instrument to the balance, the observed weight gain can reasonably be attributed to the density differences between air and  $\text{CO}_2$ . Indeed, when collection vials containing glass wool and air were purged with  $\text{CO}_2$ , they gained an average of 11.2 mg.

Interestingly, at 5 min postextraction, both types of collection vials had actually lost mass relative to their pre-extraction masses, with the vials containing glass helices losing almost 1 mg. This weight loss below the original mass was unexpected and can probably best be explained by the removal of adsorbed water from inside the collection vials, both from the packing materials (i.e., glass wool or helices) and from the collection vial itself via the warm, dry  $\text{CO}_2$  passing through the collection vials. As postextraction time increased, both types of collection vials slowly began to return to their original masses as water was readsorbed and equilibrium was re-established. By 35 min postextraction, both types of



**Figure 2.** Effect of postextraction equilibration time on collection vial weight: ground beef extraction.

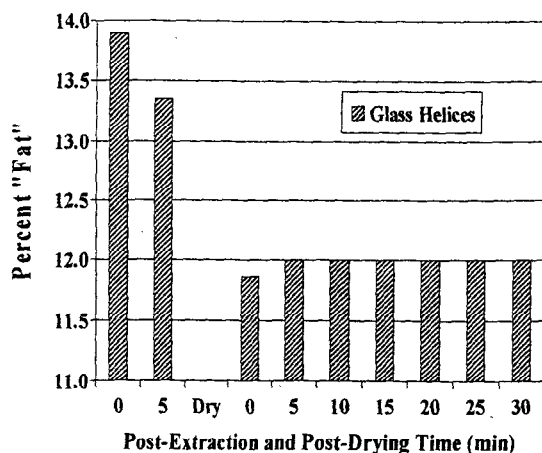


**Figure 3.** Effect of vial packing material and microwave-drying on empty collection vial weight.

collection vials had essentially returned to their pre-extraction masses.

Similarly, when the mass of collection vials after an extraction of ground beef was monitored over time, the mass decreased as postextraction time increased (Figure 2). In this case, the vials were still losing weight up to the last measurement taken at 35 min postextraction. The initial decrease in weight between 0 and 5 min is probably a result of the loss of carbon dioxide from inside the collection vial as well as from that dissolved in the fat. Carbon dioxide is soluble in triglycerides (25) and may be imbibed in the collected oil and fat after SFE. Hence, this dissolved  $\text{CO}_2$  can give high gravimetric fat values if not removed from the oil before being weighed (3). The subsequent decrease in mass with time after 5 min is probably best explained by the slow evaporation of water, which was coextracted during the  $\text{SC-CO}_2$  extraction of the fat from ground beef. In addition to its potential negative effects on lipid extraction, coextracted water may overestimate gravimetric fat determinations. Therefore, it is recommended that the collected oil be dried to remove coextracted water prior to gravimetric determinations (26).

**Effect of Microwave-Drying of Collection Vials.** The effects of microwave-drying on collection vials filled with either glass wool or glass helices, without collected fat, are shown in Figure 3. Vials with glass wool or glass helices lost ca. 2.8 and 3.3 mg, respectively. By 5 min postmicrowaving, both types of collection vials had regained all but  $\sim 1$  mg of this lost weight, and by 20–25 min both types of collection vials had essentially returned to their original mass. The decrease seen in



**Figure 4.** Effect of microwave-drying on collection vial weight: glass helices and extracted fat from ground beef.

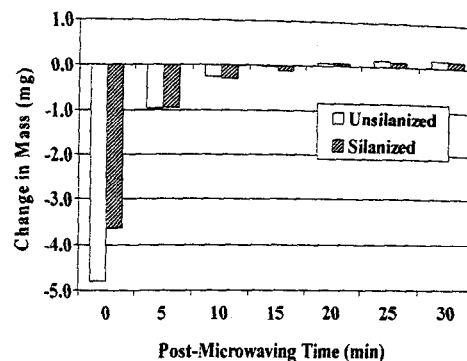
these collection vials is undoubtedly due to the removal of adsorbed water from the vials as well as the material inside the vials (i.e., glass wool or helices). The slow reabsorption of water to the glass during equilibration is responsible for return to the original mass of the vials before microwaving.

The effects of microwave-drying on collection vials with extracted/collected fat are shown in Figure 4. Initially the gravimetric percent "fat" was ~13.9% immediately after the extraction and then decreased to ~13.4% after 5 min. Immediately after microwaving for 3 min, the gravimetric fat decreased to 11.9%. By 5 min postmicrowaving, the gravimetric percent fat had increased to 12.0% and remained at this level up to the last measurement taken at 30 min postmicrowaving. The 0.1% difference observed between the 11.9% immediately after microwaving and the 12.0% at 5 min postmicrowaving is ~1.2 mg in actual weight, and 1.2 mg is very close to the weight loss seen for the collection vials without collected fat. Postmicrowaving times over 10–15 min had little impact on the calculated gravimetric percent fat for the ground beef sample. Because 1 mg of water lost during microwave-drying can have a large impact on the gravimetric "fat" determination, especially for small samples, or samples with low fat content, care should be exercised to ensure re-equilibration of microwaved collection vials.

The GC-FAME analysis of the extract without microwave-drying gave a mean ( $n = 4$ ) fat determination of 11.9% (RSD = 0.6), whereas the GC-FAME analysis of the extract with microwave-drying gave a mean fat determination of 11.7% (RSD = 1.7). Although this difference is small, it was statistically significant ( $P = 0.03$ ) (paired  $t$  test). This suggests that microwave-drying of the collected fat also may polymerize the fat, making it unavailable for subsequent GC-FAME analysis as was shown for vacuum-drying (24).

**Effect of Silanizing Collection Vials on Equilibration Time after Microwave-Drying.** The effects of microwave-drying on silanized collection vials and glass helices, without collected fat, are shown in Figure 5. In this case, the unsilanized vials lost ~4.8 mg compared to 3.6 mg for the silanized collection vials immediately after microwaving. Although the silanized collection vials had lost slightly less weight initially, after  $\geq 5$  min postmicrowaving, the two types of collection vials were essentially equal in mass.

In summary, the use of ethanol as a cosolvent modifier for SC-CO<sub>2</sub> is unnecessary for the complete extrac-



**Figure 5.** Effect of silanizing collection vials and glass helices on empty collection vial weight after microwave-drying.

tion of fat and increased the coextraction of water, causing an overestimation of the gravimetric percent fat. Oven-drying ground beef samples prior to SFE inhibits subsequent extraction of some of the fat, and oven-drying the extracted/collected fat after SFE caused only an insignificant decrease in the subsequent GC-FAME fat determination. When CO<sub>2</sub> is used without ethanol modifier, drying agents are unnecessary to retain water in the extraction cell. However, the molecular sieves used in this study adversely affected the extraction of fat. The re-equilibration of collection vials after SFE, especially the readsorption of water, in some cases required up to 30 min postextraction. Silanizing collection vials is not an effective means to decrease the loss of water from collection during microwave-drying. Microwave-drying of collection vials is an effective method for removing coextracted water from fat extracts of ground beef, and equilibration of these vials occurs within 10–15 min. However, microwave-drying caused a slight but significant decrease in the GC-FAME fat determination.

The standard of identity definitions for ground beef are given in the *U.S. Code of Federal Regulations*, Title 9, Chapter III, Part 319, Subpart B, Section 319.15. Currently, "regular" ground beef or "hamburger" must contain <30% fat, whereas "lean" and "extra-lean" must contain <10 and <5% fat, respectively. Although an SFE-based gravimetric fat determination would be sufficient to properly categorize ground beef according to these definitions or simply to provide percent fat or percent lean information, the USDA will soon issue new standards for packaged meat products, including ground beef, which will require the same nutrition information as processed foods [*Int. News Fats, Oils Relat. Mater.* 2000, 11 (August)]. The required information will include total fat, saturated fat, and mono-unsaturated fat, as well as cholesterol content. When these rules take effect, simple gravimetric fat determination will no longer suffice and GC-FAME fat analyses will be necessary. Although it would be possible to use gravimetric fat analyses in-house for quality control/assurance, it should be noted that at some point fatty acid analysis will need to be performed to provide the newly required information. Our SFE method utilizing glass helices in the collection vials is an effective method for extracting the fat and is easily followed by a BF<sub>3</sub>/methanol transesterification for subsequent GC-FAME analysis. This SFE method is quick and effective for fat extraction and uses no organic solvents.

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